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(54) **Title:** ANTI-CD73 ANTI-PD-L1 BISPECIFIC ANTIBODIES

(57) **Abstract:** Provided are bispecific antibodies capable of binding to human CD73 protein and human PD-L1 protein. These bispecific antibodies are effective in treating cancer.

ANTI-CD73 ANTI-PD-L1 BISPECIFIC ANTIBODIES

BACKGROUND

[0001] CD73, cluster of differentiation 73, is also known as 5'-nucleotidase (5'-NT) or ecto-5'-nucleotidase, is an enzyme serves to convert AMP to adenosine. CD73 catalyzes the formation of extracellular adenosine which contributes to the immunosuppressive tumor environment. CD73 is over-expressed in stromal cells and multiple types of tumor cells, as well as in Tregs, M2 Mφs and myeloid derived suppressor cells (MDSCs).

[0002] Programmed death-ligand 1 (PD-L1), also known as cluster of differentiation 274 (CD274) or B7 homolog 1 (B7-H1), is a 40kDa type 1 transmembrane protein believed to play a major role in suppressing the immune system during particular events such as pregnancy, tissue allografts, autoimmune disease and other disease states such as hepatitis. The binding of PD-L1 to PD-1 or B7.1 transmits an inhibitory signal which reduces the proliferation of CD8+ T cells at the lymph nodes and supplementary to that PD-1 is also able to control the accumulation of foreign antigen specific T cells in the lymph nodes through apoptosis which is further mediated by a lower regulation of the gene Bcl-2.

[0003] Preclinical evidence shows that CD73 inhibition prevented adenosine-mediated lymphocyte suppression, increased the activity of CD8+ effector cells, and reduced both MDSCs and Tregs. It has been shown that upregulation of PD-L1 may allow cancers to evade the host immune system. An analysis of tumor specimens from patients with renal cell carcinoma found that high tumor expression of PD-L1 was associated with increased tumor aggressiveness and an increased risk of death. Many PD-L1 inhibitors are in development as immuno-oncology therapies and are showing good results in clinical trials.

[0004] Bispecific antibodies targeting both the CD73 and PD-L1 proteins have been proposed, but development of bispecific antibodies with good stability and activity has been proved to be challenging.

SUMMARY

[0005] The present disclosure provides bispecific antibodies having binding specificities to both CD73 and PD-L1 proteins. As shown in the experimental examples, these bispecific antibodies exhibited high binding affinity to both proteins and were effective in inhibiting

CD73 enzymatic activities and blocking PD-L1 to PD-1 binding, resulting in T-cell activation.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] FIG. 1 illustrates the structures of four different bispecific antibodies prepared.

[0007] FIG. 2 shows the binding results to soluble CD73 protein.

[0008] FIG. 3 shows the binding results to soluble PD-L1 protein.

[0009] FIG. 4 shows the binding results to cell surface CD73 protein.

[0010] FIG. 5 shows the binding results to cell surface PD-L1 protein.

[0011] FIG. 6 shows that the bispecific antibody blocked soluble CD73 enzymatic activitiy.

[0012] FIG. 7 shows that the bispecific antibody blocked cell surface CD73 enzymatic activitiy.

[0013] FIG. 8 shows that the bispecific antibody blocked cell surface PD-L1 to PD-L1 binding, reslting in T-cell activation.

DETAILED DESCRIPTION

Definitions

[0014] As used herein, an “antibody” or “antigen-binding polypeptide” refers to a polypeptide or a polypeptide complex that specifically recognizes and binds to an antigen. An antibody can be a whole antibody and any antigen binding fragment or a single chain thereof. Thus the term “antibody” includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule having biological activity of binding to the antigen. Examples of such include, but are not limited to a complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework (FR) region, or any portion thereof, or at least one portion of a binding protein.

[0015] The terms “antibody fragment” or “antigen-binding fragment”, as used herein, is a portion of an antibody such as $F(ab')_2$, $F(ab)_2$, Fab', Fab, Fv, scFv and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. The term “antibody fragment” includes aptamers, spiegelmers, and diabodies. The term “antibody fragment” also includes any synthetic or genetically engineered protein that acts like an antibody by binding to a specific antigen to form a complex.

[0016] A “single-chain variable fragment” or “scFv” refers to a fusion protein of the variable regions of the heavy (V_H) and light chains (V_L) of immunoglobulins. In some aspects, the regions are connected with a short linker peptide of ten to about 25 amino acids. The linker can be rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the V_H with the C-terminus of the V_L, or vice versa. This protein retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of the linker. ScFv molecules are known in the art and are described, *e.g.*, in US patent 5,892,019.

[0017] By “specifically binds” or “has specificity to,” it is generally meant that an antibody binds to an epitope via its antigen-binding domain, and that the binding entails some complementarity between the antigen-binding domain and the epitope. According to this definition, an antibody is said to “specifically bind” to an epitope when it binds to that epitope, via its antigen-binding domain more readily than it would bind to a random, unrelated epitope. The term “specificity” is used herein to qualify the relative affinity by which a certain antibody binds to a certain epitope. For example, antibody “A” may be deemed to have a higher specificity for a given epitope than antibody “B,” or antibody “A” may be said to bind to epitope “C” with a higher specificity than it has for related epitope “D.”

[0018] As used herein, the terms “treat” or “treatment” refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the progression of cancer. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. “Treatment” can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in

need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

Anti-CD73 Anti-PD-L1 Bispecific Antibodies

[0019] The present disclosure provides anti-CD73 anti-PD-L1 bispecific antibodies with high affinity and inhibitory activity to both human CD73 and PD-L1 proteins. The antibodies can bind effectively to both soluble and cell surfaces CD73 and PD-L1. Such bindings were capable of blocking CD73 enzymatic activities and blocking PD-L1 binding to PD-1, resulting in TCR activation and increased IL-2 production.

[0020] In accordance with one embodiment of the present disclosure, provided is a bispecific antibody that has binding specificities to both human CD73 and PD-L1 protein. In one embodiment, the bispecific antibody has an anti-CD73 portion that includes CDR regions as shown in **Table 1**.

Table 1. Anti-CD73 CDRs

Name	Sequences	SEQ ID NO:
VH CDR1	**SGYYWN**	1
VH CDR2	**YINYGGSNGYNPSLKS**	2
VH CDR3	**DYDAYYEALDD**	3
VL CDR1	**RASSRVNYMH**	4
VL CDR2	**ATSNLAS**	5
VL CDR3	**QQWSSNPPT**	6

[0021] In one embodiment, the bispecific antibody has an anti-PD-L1 portion that includes CDR regions as shown in **Table 2**.

Table 2. Anti-PD-L1 CDRs

Name	Sequence	SEQ ID NO:
VH CDR1	**SYDMS**	7
VH CDR2	**TISDAGGYIYYRDSVKG**	8
VH CDR3	**ELPWRYALDY**	9

VL CDR1	**KASQDVTPAVA**	10
VL CDR2	**STSSRYT**	11
VL CDR3	**QQHYTTPLT**	12

[0022] An example of the heavy chain variable region (VH) of the anti-CD73 portion is SEQ ID NO:21. An example of the light chain variable region (VL) of the anti-CD73 portion is SEQ ID NO:22.

[0023] An example of the heavy chain variable region (VH) of the anti-PD-L1 portion is SEQ ID NO:23. An example of the light chain variable region (VL) of the anti-PD-L1 portion is SEQ ID NO:24.

[0024] Non-limiting examples of the structures of the bispecific antibody are illustrated in **FIG. 1**. In one example, the bispecific antibody includes a Fab portion linked to a single-chain variable fragment (scFv). As illustrated in FIG. 1, both antigen-binding domains of the Fab portion can target one protein, whereas the scFv has specificity to the other protein.

[0025] Other structures are also contemplated. For example, the bispecific antibody can be a heterodimer, including one heavy chain-light chain pair targeting CD73 and the other heavy chain-light chain pair targeting PD-L1. In some embodiments, one of the heavy chain-light chain pairs can be replaced by a scFv, forming a Fab-scFv structure. In some embodiments, one of the heavy chain-light chain pairs can be replaced by a nanobody (also known as single-domain antibody or VHH), forming a Fab-VHH structure. In some embodiments, both chain-light chain pairs can be replaced by other forms of antigen-binding domains, such as scFv and VHH. In some embodiments, the bispecific antibody can be a single chain, such as having two scFv or VHH connected to each other, or a scFv connected to a VHH, without limitation.

[0026] In the Fab/scFv examples of FIG. 1, in some embodiments, the Fab fragments have specificity to CD73 and the scFv fragment has specificity to PD-L1. In some embodiments, the Fab fragments have specificity to PD-L1 and the scFv fragment has specificity to CD73.

[0027] A peptide linker can be used to connect the Fab portion (or to the Fc if included) and the scFv portion of the bispecific antibody. Likewise, a linker can be used to connect the

heavy chain variable region and the light chain variable region within the scFv. Two examples of such peptide linkers are provided in SEQ ID NO:13 and 14.

[0028] Example complete sequences of the heavy chains and light chains of the bispecific antibodies are also provided. In one embodiment, the antibody includes two heavy chains each comprising the amino acid sequence of SEQ ID NO:15 and two light chains each comprising the amino acid sequence of SEQ ID NO:16. In one embodiment, the antibody includes two heavy chains each comprising the amino acid sequence of SEQ ID NO:17 and two light chains each comprising the amino acid sequence of SEQ ID NO:16. In one embodiment, the antibody includes two heavy chains each comprising the amino acid sequence of SEQ ID NO:18 and two light chains each comprising the amino acid sequence of SEQ ID NO:19. In one embodiment, the antibody includes two heavy chains each comprising the amino acid sequence of SEQ ID NO:20 and two light chains each comprising the amino acid sequence of SEQ ID NO:19. It will also be understood by one of ordinary skill in the art that antibodies as disclosed herein may be modified such that they vary in amino acid sequence from the naturally occurring binding polypeptide from which they were derived. For example, a polypeptide or amino acid sequence derived from a designated protein may be similar, *e.g.,* have a certain percent identity to the starting sequence, *e.g.,* it may be 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to the starting sequence.

[0029] In certain embodiments, the antibody comprises an amino acid sequence or one or more moieties not normally associated with an antibody. Exemplary modifications are described in more detail below. For example, an antibody of the disclosure may comprise a flexible linker sequence, or may be modified to add a functional moiety (*e.g.*, PEG, a drug, a toxin, or a label).

[0030] Antibodies, variants, or derivatives thereof of the disclosure include derivatives that are modified, *i.e.*, by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from binding to the epitope. For example, but not by way of limitation, the antibodies can be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation,

metabolic synthesis of tunicamycin, etc. Additionally, the antibodies may contain one or more non-classical amino acids.

[0031] In some embodiments, the antibodies may be conjugated to therapeutic agents, prodrugs, peptides, proteins, enzymes, viruses, lipids, biological response modifiers, pharmaceutical agents, or PEG.

[0032] The antibodies may be conjugated or fused to a therapeutic agent, which may include detectable labels such as radioactive labels, an immunomodulator, a hormone, an enzyme, an oligonucleotide, a photoactive therapeutic or diagnostic agent, a cytotoxic agent, which may be a drug or a toxin, an ultrasound enhancing agent, a non-radioactive label, a combination thereof and other such agents known in the art.

[0033] The antibodies can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antigen-binding polypeptide is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

Polynucleotides Encoding the Antibodies and Methods of Preparing the Antibodies

[0034] The present disclosure also provides isolated polynucleotides or nucleic acid molecules encoding the antibodies, variants or derivatives thereof of the disclosure. The polynucleotides of the present disclosure may encode the entire heavy and light chain variable regions of the antigen-binding polypeptides, variants or derivatives thereof on the same polynucleotide molecule or on separate polynucleotide molecules. Additionally, the polynucleotides of the present disclosure may encode portions of the heavy and light chain variable regions of the antigen-binding polypeptides, variants or derivatives thereof on the same polynucleotide molecule or on separate polynucleotide molecules.

[0035] Methods of making antibodies are well known in the art and described herein. In certain embodiments, both the variable and constant regions of the antigen-binding polypeptides of the present disclosure are fully human. Fully human antibodies can be made using techniques described in the art and as described herein. For example, fully human antibodies against a specific antigen can be prepared by administering the antigen to a

transgenic animal which has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled. Exemplary techniques that can be used to make such antibodies are described in U.S. patents: 6,150,584; 6,458,592; 6,420,140 which are incorporated by reference in their entireties.

Treatment Methods

[0036] As described herein, the antibodies, variants or derivatives of the present disclosure may be used in certain treatment and diagnostic methods.

[0037] Accordingly, in some embodiments, provided are methods for treating a cancer in a patient in need thereof. The method, in one embodiment, entails administering to the patient an effective amount of an antibody of the present disclosure. In some embodiments, at least one of the cancer cells (e.g., stromal cells) in the patient over-expresses CD73. In some embodiments, at least one of the cancer cells (e.g., stromal cells) in the patient over-expresses PD-L1.

[0038] Non-limiting examples of cancers include bladder cancer, breast cancer, colorectal cancer, endometrial cancer, esophageal cancer, head and neck cancer, kidney cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, pancreatic cancer, prostate cancer, and thyroid cancer.

[0039] Cellular therapies, and more specifically chimeric antigen receptor (CAR) T-cell therapies, are also provided in the present disclosure. A suitable T cell can be used, that is put in contact with a bispecific antibody of the present disclosure (or alternatively engineered to express a bispecific antibody of the present disclosure). Upon such contact or engineering, the T cell can then be introduced to a cancer patient in need of a treatment. The cancer patient may have a cancer of any of the types as disclosed herein. The T cell can be, for instance, a tumor-infiltrating T lymphocyte, a CD4+ T cell, a CD8+ T cell, or the combination thereof, without limitation.

[0040] In some embodiments, the T cell was isolated from the cancer patient him- or her-self. In some embodiments, the T cell was provided by a donor or from a cell bank. When the T cell is isolated from the cancer patient, undesired immune reactions can be minimized.

[0041] Additional diseases or conditions associated with increased cell survival, that may be treated, prevented, diagnosed and/or prognosed with the antibodies or variants, or derivatives thereof of the disclosure include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (*e.g.*, acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (*e.g.*, chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (*e.g.*, Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyo sarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma and retinoblastoma.

[0042] A specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the particular antibodies, variant or derivative thereof used, the patient's age, body weight, general health, sex, and diet, and the time of administration, rate of excretion, drug combination, and the severity of the particular disease being treated. Judgment of such factors by medical caregivers is within the ordinary skill in the art. The amount will also depend on the individual patient to be treated, the route of administration, the type of formulation, the characteristics of the compound used, the severity of the disease, and the desired effect. The amount used can be determined by pharmacological and pharmacokinetic principles well known in the art.

[0043] Methods of administration of the antibodies, variants or include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The antigen-binding polypeptides or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Thus, pharmaceutical compositions containing the antigen-binding polypeptides of the disclosure may be administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray.

[0044] The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intra-articular injection and infusion.

[0045] Administration can be systemic or local. In addition, it may be desirable to introduce the antibodies of the disclosure into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0046] It may be desirable to administer the antigen-binding polypeptides or compositions of the disclosure locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, *e.g.*, in conjunction, with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the disclosure, care must be taken to use materials to which the protein does not absorb.

[0047] The amount of the antibodies of the disclosure which will be effective in the treatment, inhibition and prevention of an inflammatory, immune or malignant disease, disorder or condition can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose

to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease, disorder or condition, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[0048] As a general proposition, the dosage administered to a patient of the antigen-binding polypeptides of the present disclosure is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight, between 0.1 mg/kg and 20 mg/kg of the patient's body weight, or 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the disclosure may be reduced by enhancing uptake and tissue penetration (*e.g.*, into the brain) of the antibodies by modifications such as, for example, lipidation.

[0049] The methods for treating an infectious or malignant disease, condition or disorder comprising administration of an antibody, variant, or derivative thereof of the disclosure are typically tested *in vitro*, and then *in vivo* in an acceptable animal model, for the desired therapeutic or prophylactic activity, prior to use in humans. Suitable animal models, including transgenic animals, are well known to those of ordinary skill in the art. For example, *in vitro* assays to demonstrate the therapeutic utility of antigen-binding polypeptide described herein include the effect of an antigen-binding polypeptide on a cell line or a patient tissue sample. The effect of the antigen-binding polypeptide on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art, such as the assays disclosed elsewhere herein. In accordance with the disclosure, *in vitro* assays which can be used to determine whether administration of a specific antigen-binding polypeptide is indicated, include *in vitro* cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

[0050] Various delivery systems are known and can be used to administer an antibody of the disclosure or a polynucleotide encoding an antibody of the disclosure, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (*see, e.g.*, Wu and Wu, 1987, *J. Biol. Chem.* 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, etc.

[0051] In a further embodiment, the compositions of the disclosure are administered in combination with an antineoplastic agent, an antiviral agent, antibacterial or antibiotic agent or antifungal agents. Any of these agents known in the art may be administered in the compositions of the current disclosure.

[0052] In another embodiment, compositions of the disclosure are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the compositions of the disclosure include, but are not limited to, antibiotic derivatives (*e.g.*, doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (*e.g.*, tamoxifen); antimetabolites (*e.g.*, fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (*e.g.*, carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (*e.g.*, medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (*e.g.*, mephalen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids and combinations (*e.g.*, bethamethasone sodium phosphate); and others (*e.g.*, dicarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, and etoposide).

[0053] In an additional embodiment, the compositions of the disclosure are administered in combination with cytokines. Cytokines that may be administered with the compositions of the disclosure include, but are not limited to, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL-15, anti-CD40, CD40L, and TNF-α.

[0054] In additional embodiments, the compositions of the disclosure are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

Compositions

[0055] The present disclosure also provides pharmaceutical compositions. Such compositions comprise an effective amount of an antibody, and an acceptable carrier. In some embodiments, the composition further includes a second anticancer agent (e.g., an immune checkpoint inhibitor).

[0056] In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. Further, a "pharmaceutically acceptable carrier" will generally be a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

[0057] The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents such as acetates, citrates or phosphates. Antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; and agents for the adjustment of tonicity such as sodium chloride or dextrose are also envisioned. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences by E. W. Martin, incorporated herein by reference. Such compositions will contain a therapeutically effective amount of the antigen-binding polypeptide, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0058] In an embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0059] The compounds of the disclosure can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

EXAMPLES

Example 1. Preparation of CD73/PD-L1 bispecific antibodies

[0060] This example describes the preparation of four different bispecific antibodies having specificities to both human CD73 and PD-L1 proteins. The activities of one of them were then tested *in vitro*.

[0061] The structures of the four bispecific antibodies are illustrated in **FIG. 1**. Each of them included an IgG1 Fab fragment, connected through a linker ($(G4S)_3$, SEQ ID NO:13, or R64C, SEQ ID NO:14), to a single-chain variable fragment (scFv). The anti-CD73 regions are referred to as 309 and the anti-PD-L1 regions are referred to as H12. In panels A and B, the bispecific antibodies included 309 in the Fab portion and H12 in the scFv portion. In panels C and D, the bispecific antibodies included H12 in the Fab portion and 309 in the scFv portion.

[0062] In panels A and C, the linkers between the Fab and the scFv were the $(G4S)_3$ linker (SEQ ID NO:13), and the scFv used the R64C linker (SEQ ID NO:14) between the heavy chain variable region and the light chain variable region. In panels B and D, the linkers between the Fab and the scFv were the R64C linker (SEQ ID NO:14), and the scFv used the $(G4S)_3$ linker (SEQ ID NO:13) between the heavy chain variable region and the light chain variable region.

[0063] The $(G4S)_3$ linker has the following amino acid sequence: GGGGSGGGGSGGGGS (SEQ ID NO:13). The R64C linker has the following amino acid sequence: PCEPTKTEREEQEEKEKEKKKEEGGRGTNRTTAPATTAKALSGEAQPQATPVSSAQA KPSEPWR (SEQ ID NO:14).

[0064] The amino acid sequences of the antibodies and fragments are listed in the table below.

Table 3. Amino acid sequences

Name (SEQ ID NO:)	Sequence
A-heavy chain (15)	>309 VH EVQLQESGPGLVKPSETLSLTCAVSGYSITSGYYWNWIRQPPGKKLEWMG YINYGGSNGYNPSLKSRITISRDTSKNQFSLKLSSVTAADTAVYYCARDY DAYYEALDDWGQGTTVTVSS >IgG1 CH1-CH2-CH3 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK >$(G4S)_3$ linker GGGGSGGGGSGGGGS >H12 VH EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKSLEWVAT ISDAGGYIYYRDSVKGRFTISRDNAKNSLYLQMNSLRDEDTAVYICAREL PWRYALDYWGQGTTVTVSS >$(G4S)_3$ linker GGGGSGGGGSGGGGS >H12 VL DIQMTQSPSSLSASVGDRVTITCKASQDVTPAVAWYQQKPGKAPKLLIYS TSSRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQHYTTPLTFGQ GTKLEIK
A-light chain (16)	>309 VL EIVLSQSPATLSLSPGERATLSCRASSRVNYMHWYQQKPGQSPRPWISAT

	SNLASGVPARFSGSGSGTSYTLTISSLEPEDFAVYYCQQWSSNPPTFGGG TKVEIK >IgG1 CL RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC
B-heavy chain (17)	>309 VH EVQLQESGPGLVKPSETLSLTCAVSGYSITSGYYWNWIRQPPGKKLEWMG YINYGGSNGYNPSLKSRITISRDTSKNQFSLKLSSVTAADTAVYYCARDY DAYYEALDDWGQGTTVTVSS >IgG1 CH1-CH2-CH3 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK >R64C linker PCEPTKTEREEQEEKEKEKKKEEGGRGTNRTTAPATTAKALSGEAQPQAT PVSSAQAKPSEPWR >H12 VH EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKSLEWVAT ISDAGGYIYYRDSVKGRFTISRDNAKNSLYLQMNSLRDEDTAVYICAREL PWRYALDYWGQGTTVTVSS >(G4S)$_3$ linker GGGGSGGGGSGGGGS >H12 VL DIQMTQSPSSLSASVGDRVTITCKASQDVTPAVAWYQQKPGKAPKLLIYS TSSRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQHYTTPLTFGQ GTKLEIK
B-light chain (16)	>309 VL EIVLSQSPATLSLSPGERATLSCRASSRVNYMHWYQQKPGQSPRPWISAT SNLASGVPARFSGSGSGTSYTLTISSLEPEDFAVYYCQQWSSNPPTFGGG TKVEIK >IgG1 CL RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC
C-heavy chain (18)	>H12 VH EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKSLEWVAT ISDAGGYIYYRDSVKGRFTISRDNAKNSLYLQMNSLRDEDTAVYICAREL PWRYALDYWGQGTTVTVSS >IgG1 CH1-CH2-CH3 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW

	QQGNVFSCSVMHEALHNHYTQKSLSLSPGK >(G4S)$_3$ linker GGGGSGGGGSGGGGS >309 VH EVQLQESGPGLVKPSETLSLTCAVSGYSITSGYYWNWIRQPPGKKLEWMG YINYGGSNGYNPSLKSRITISRDTSKNQFSLKLSSVTAADTAVYYCARDY DAYYEALDDWGQGTTVTVSS >(G4S)$_3$ linker GGGGSGGGGSGGGGS >309 VL EIVLSQSPATLSLSPGERATLSCRASSRVNYMHWYQQKPGQSPRPWISAT SNLASGVPARFSGSGSGTSYTLTISSLEPEDFAVYYCQQWSSNPPTFGGG TKVEIK
C-light chain (19)	>H12 VL DIQMTQSPSSLSASVGDRVTITCKASQDVTPAVAWYQQKPGKAPKLLIYS TSSRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQHYTTPLTFGQ GTKLEIK >IgG1 CL RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC
D-heavy chain (20)	>H12 VH EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKSLEWVAT ISDAGGYIYYRDSVKGRFTISRDNAKNSLYLQMNSLRDEDTAVYICAREL PWRYALDYWGQGTTVTVSS >IgG1 CH1-CH2-CH3 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK >R64C linker PCEPTKTEREEQEEKEKEKKKEEGGRGTNRTTAPATTAKALSGEAQPQAT PVSSAQAKPSEPWR >309 VH EVQLQESGPGLVKPSETLSLTCAVSGYSITSGYYWNWIRQPPGKKLEWMG YINYGGSNGYNPSLKSRITISRDTSKNQFSLKLSSVTAADTAVYYCARDY DAYYEALDDWGQGTTVTVSS >(G4S)$_3$ linker GGGGSGGGGSGGGGS >309 VL EIVLSQSPATLSLSPGERATLSCRASSRVNYMHWYQQKPGQSPRPWISAT SNLASGVPARFSGSGSGTSYTLTISSLEPEDFAVYYCQQWSSNPPTFGGG TKVEIK
D-light chain (19)	>H12 VL DIQMTQSPSSLSASVGDRVTITCKASQDVTPAVAWYQQKPGKAPKLLIYS

	TSSRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQHYTTPLTFGQ GTKLEIK >IgG1 CL RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC
309 heavy chain variable region (21)	EVQLQESGPGLVKPSETLSLTCAVSGYSITSGYYWNWIRQPPGKKLEWMG YINYGGSNGYNPSLKSRITISRDTSKNQFSLKLSSVTAADTAVYYCARDY DAYYEALDDWGQGTTVTVSS
309 light chain variable region (22)	EIVLSQSPATLSLSPGERATLSCRASSRVNYMHWYQQKPGQSPRPWISAT SNLASGVPARFSGSGSGTSYTLTISSLEPEDFAVYYCQQWSSNPPTFGGG TKVEIK
H12 heavy chain variable region (23)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKSLEWVAT ISDAGGYIYYRDSVKGRFTISRDNAKNSLYLQMNSLRDEDTAVYICAREL PWRYALDYWGQGTTVTVSS
H12 light chain variable region (24)	DIQMTQSPSSLSASVGDRVTITCKASQDVTPAVAWYQQKPGKAPKLLIYS TSSRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQHYTTPLTFGQ GTKLEIK

[0065] The antibodies were purified from 200ml transiently transfected supernatant of the HEK293F cells by Protein A affinity column. The purity of the antibodies were tested with HPLC and SDS-PAGE.

Example 2. ELISA binding to human CD73 and PD-L1 proteins

[0066] This example shows the ELISA binding assay results for structure A (**FIG. 1**) of the anti-CD73/PD-L1 bispecific antibodies. The antibody is also referred simply as 309-H12. The monospecific antibodies, hu309 (monospecific anti-CD73 antibody with the 309 VH and 309 VL) and H12(PDL1) (monospecific anti-PD-L1 antibody with the H12 VH and H12 VL), were used as control.

[0067] Plated were coated with 100μl of 1μg/ml human CD73 protein overnight. The samples were blocked with 1%BSA in PBST for 1hr, washed with PBST 3x. Binding was tested with 100μl of serial diluted antibodies (309-H12 or hu309) from 1000pM by 3-fold, RT for 30min, washed with PBST 3x. Binding with anti-hu Fc HRP was at 1:10000 in PBST, RT for 30min, wash with PBST 3x. 100μl of TMB and 100μl of HCl were then added.

[0068] As shown in **FIG. 2**, the bispecific antibody 309-H12 had similar binding activities as hu309 in binding to CD73.

[0069] Using the same procedure, the binding activities of 309-H12 and H12(PDL1) to the human PD-L1 protein were tested with ELISA. Although the binding activities of the bispecific antibody was lower than the monospecific one, both of the activities were high (**FIG. 3**).

Example 3. Cell-based binding of the bispecific antibody

[0070] This example shows the cell-based binding assay results for 309-H12. Like in Example 2, monospecific antibodies hu309 and H12(PDL1) were used as control.

[0071] A375 cells that constitutively expressed CD73 on the surface were used in this example. $1X10^5$ A375 cells/well were suspended in FACS buffer. Antibodies diluted from 10nM by 2-fold, 100μl into each well, 4°C for 30min. The samples were washed with FACS buffer for 1X. AF633-anti hu Fc was added for binding for 30min at 4°C, and washed with FACS buffer for 1X.

[0072] As shown in **FIG. 4**, the bispecific antibody 309-H12 had similar binding activities as hu309 in binding to cell surface CD73.

[0073] Using the same procedure, the binding activities of 309-H12 and H12(PDL1) to the human PD-L1 protein from PD-L1 positive Raji cells. Although the binding activities of the bispecific antibody was lower than the monospecific one, both of the activities were high (**FIG. 5**).

Example 4. Blocking of CD73 activity by the bispecific antibody

[0074] This example shows that the 309-H12 antibody was as effective as the monospecific hu309 antibody in blocking CD73 enzymatic activities.

[0075] A sample containing 400pM (0.0252μg/ml) CD73 was used. Antibodies were diluted from 4nM (hu309 0.6μg/ml, 309-H12 0.8μg/ml) by 2 fold. CD73 and each antibody were pre-incubated for 30min at 37°C, and AMP(100μM) and ATP(100μM) and incubated for 6hrs at 37°C. Equal volumes of cell titerglo was then added to the samples.

[0076] As shown in **FIG. 6**, the bispecific antibody 309-H12 had similar ability as hu309 in blocking the enzymatic activity of soluble CD73.

[0077] Similar results were obtained for these antibodies in blocking cell surface CD73 enzymatic activity (**FIG. 7**). The A375 cells were used in this test. The samples contained 50µl of 15000 A375 cells in DMEM, and the antibodies were serial diluted from 160nM(hu309 240µg/ml, 309-H12 320µg/ml), 25µl per well, 37°C for 30min. AMP:800µM 25µl per well, 17~24hr. After 17~24hr, 50µl of cell supernatants were transferred into 96-well black plate, and 50µl of 40µM ATP were added. 100µl of cell titerglo was then added into black plate.

Example 5. T-cell activation

[0078] This example shows that the 309-H12 bispecific antibody was able to block PD-L1 to PD-1 binding, resulting in TCR activation and increased IL-2 production.

[0079] PD-1 effector cells which are Jurkat T cells expressing human PD-1 and IL-2 reporter driven by an NFAF response element and PD-L1+ cells which are Raji cells expressing human PD-L1 were used in this assay. The two cell types were co-cultured in the presence of TCR stimulation (SEE superantigen), the PD-1/PD-L1 interaction inhibited TCR signaling and NFAT-mediated IL-2 production. Addition of PD-L1 antibody H12 or the bispecific antibody 309-H12 blocked the PD-1/PD-L1 interaction, thereby releasing the inhibitory signal and resulting in TCR activation and increased IL-2 production (**FIG. 8**).

* * *

[0080] The present disclosure is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the disclosure, and any compositions or methods which are functionally equivalent are within the scope of this disclosure. It will be apparent to those skilled in the art that various modifications and variations can be made in the methods and compositions of the present disclosure without departing from the spirit or scope of the disclosure. Thus, it is intended that the present disclosure cover the modifications and variations of this disclosure provided they come within the scope of the appended claims and their equivalents.

[0081] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

CLAIMS

What is claimed is:

1. A bispecific antibody comprising an anti-CD73 portion and an anti-PD-L1 portion,

wherein the anti-CD73 portion has binding specificity to a human CD73 protein and comprises a heavy chain variable region comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO:1, a CDRH2 comprising the amino acid sequence of SEQ ID NO:2, and a CDRH3 comprising the amino acid sequence of SEQ ID NO:3, and a light chain variable region comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO:4, a CDRL2 comprising the amino acid sequence of SEQ ID NO:5, and a CDRL3 comprising the amino acid sequence of SEQ ID NO:6, and

wherein the anti-PD-L1 portion has binding specificity to a human PD-L1 protein and comprises a heavy chain variable region comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO:7, a CDRH2 comprising the amino acid sequence of SEQ ID NO:8, and a CDRH3 comprising the amino acid sequence of SEQ ID NO:9, and a light chain variable region comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO:10, a CDRL2 comprising the amino acid sequence of SEQ ID NO:11, and a CDRL3 comprising the amino acid sequence of SEQ ID NO:12.

2. The bispecific antibody of claim 1, wherein the anti-CD73 portion comprises a Fab fragment.

3. The bispecific antibody of claim 2, wherein the anti-PD-L1 portion comprises a single-chain variable fragment (scFv).

4. The bispecific antibody of claim 1, wherein the anti-CD73 portion comprises a single-chain variable fragment (scFv).

5. The bispecific antibody of claim 4, wherein the anti-PD-L1 portion comprises a Fab fragment.

6. The bispecific antibody of claim 3 or 5, comprising a first peptide linker between the Fab and the scFv.

7. The bispecific antibody of claim 6, wherein the first peptide linker comprises SEQ ID NO:13 or SEQ ID NO:14.

8. The bispecific antibody of claim 6, wherein the scFv comprises a second peptide linker between the heavy chain variable region and the light chain variable region.

9. The bispecific antibody of claim 8, wherein the second peptide linker comprises SEQ ID NO:13 or SEQ ID NO:14.

10. The bispecific antibody of claim 1, wherein the anti-CD73 portion and the anti-PD-L1 portion form a heterodimer.

11. The bispecific antibody of claim 10, wherein the anti-CD73 portion comprises a Fab fragment, a single-chain variable fragment (scFv) or a single-domain antibody (VHH).

12. The bispecific antibody of claim 10 or 11, wherein the anti-PD-L1 portion comprises a Fab fragment, a single-chain variable fragment (scFv) or a single-domain antibody (VHH).

13. The bispecific antibody of claim 12, wherein the anti-CD73 and the anti-PD-L1 portions each comprises a Fab fragment.

14. The bispecific antibody of any one of claims 1-13, further comprising a Fc fragment.

15. The bispecific antibody of any one of claims 1-14, wherein the heavy chain variable region of the anti-CD73 portion comprises the amino acid sequence of SEQ ID NO:21 and the light chain variable region of the anti-CD73 portion comprises the amino acid sequence of SEQ ID NO:22.

16. The bispecific antibody of any one of claims 1-15, wherein the heavy chain variable region of the anti-PD-L1 portion comprises the amino acid sequence of SEQ ID NO:23 and the light chain variable region of the anti-PD-L1 portion comprises the amino acid sequence of SEQ ID NO:24.

17. The bispecific antibody of claim 1, comprising two heavy chains each comprising the amino acid sequence of SEQ ID NO:15 and two light chains each comprising the amino acid sequence of SEQ ID NO:16.

18. The bispecific antibody of claim 1, comprising two heavy chains each comprising the amino acid sequence of SEQ ID NO:17 and two light chains each comprising the amino acid sequence of SEQ ID NO:16.

19. The bispecific antibody of claim 1, comprising two heavy chains each comprising the amino acid sequence of SEQ ID NO:18 and two light chains each comprising the amino acid sequence of SEQ ID NO:19.

20. The bispecific antibody of claim 1, comprising two heavy chains each comprising the amino acid sequence of SEQ ID NO:20 and two light chains each comprising the amino acid sequence of SEQ ID NO:19.

21. A composition comprising the antibody of any one of claims 1-20 and a pharmaceutically acceptable carrier.

22. A polynucleotide encoding a heavy chain of the antibody of any one of claims 1-20.

23. A cell comprising one or more polynucleotide encoding the antibody of any one of claims 1-20.

24. A method of treating cancer in a patient in need thereof, comprising administering to the patient the antibody of any one of claims 1-20.

25. Use of the antibody of any one of claims 1-20 for the manufacture of a medicament for the treatment of cancer.

26. The method of claim 24 or the use of claim 25, wherein the cancer is selected from the group consisting of bladder cancer, breast cancer, colorectal cancer, endometrial cancer, esophageal cancer, head and neck cancer, kidney cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, pancreatic cancer, prostate cancer, and thyroid cancer.

27. A method of treating cancer in a patient in need thereof, comprising:

(a) treating a T cell, *in vitro*, with the antibody of any one of claims 1-20; and

(b) administering the treated T cell to the patient.

28. The method of claim 26, further comprising, prior to step (a), isolating the T cell from an individual.

29. The method of claim 27, wherein the T cell is isolated from the patient.

30. The method of any one of claim 26-28, wherein the T cell is a tumor-infiltrating T lymphocyte, a CD4+ T cell, a CD8+ T cell, or the combination thereof.

31. Use of a composition for the manufacture of a medicament for the treatment of cancer, wherein the composition comprises a T cell treated the antibody of any one of claims 1-20.

32. The use of claim 31, wherein the T cell is a tumor-infiltrating T lymphocyte, a CD4+ T cell, a CD8+ T cell, or the combination thereof.

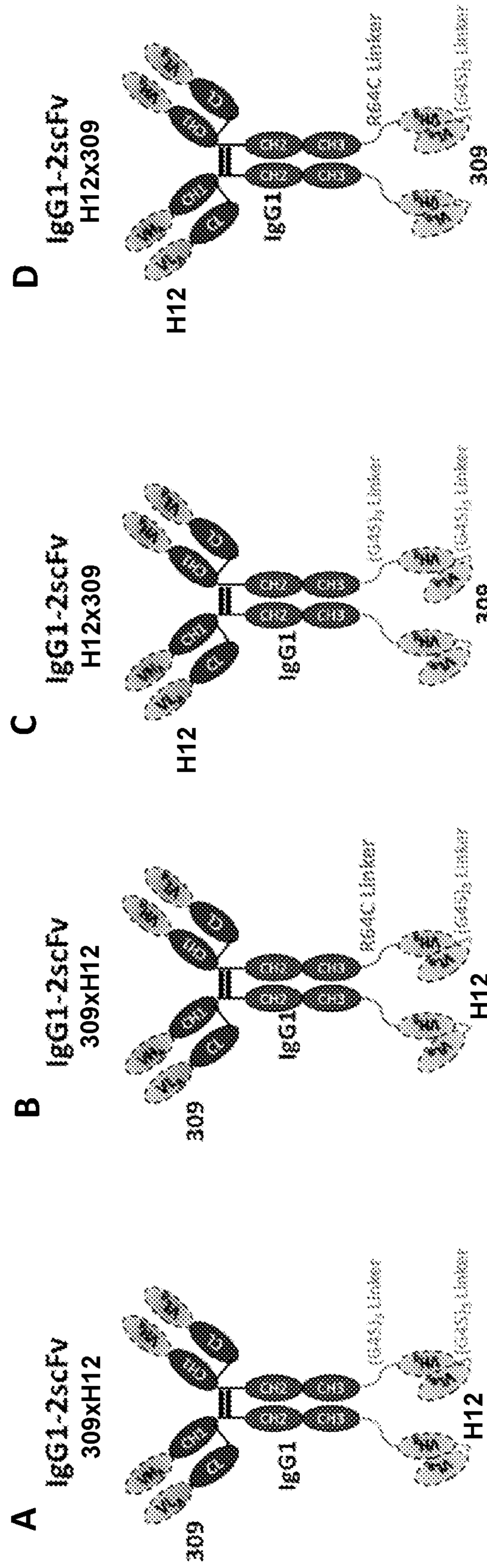


FIG. 1

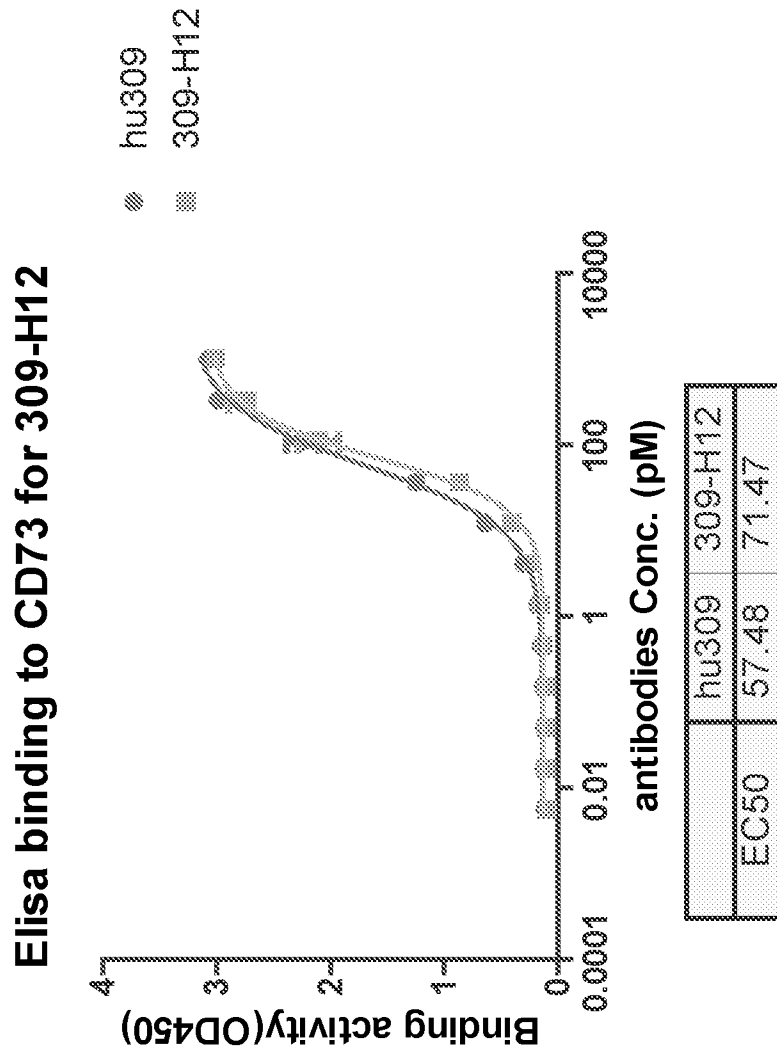
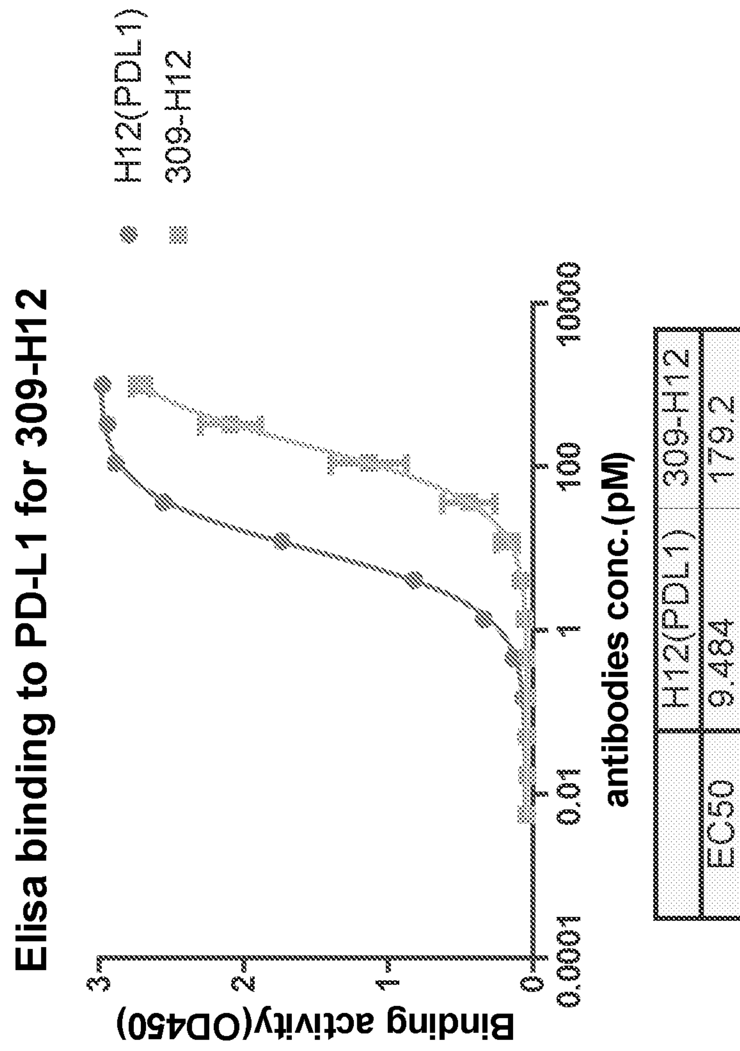
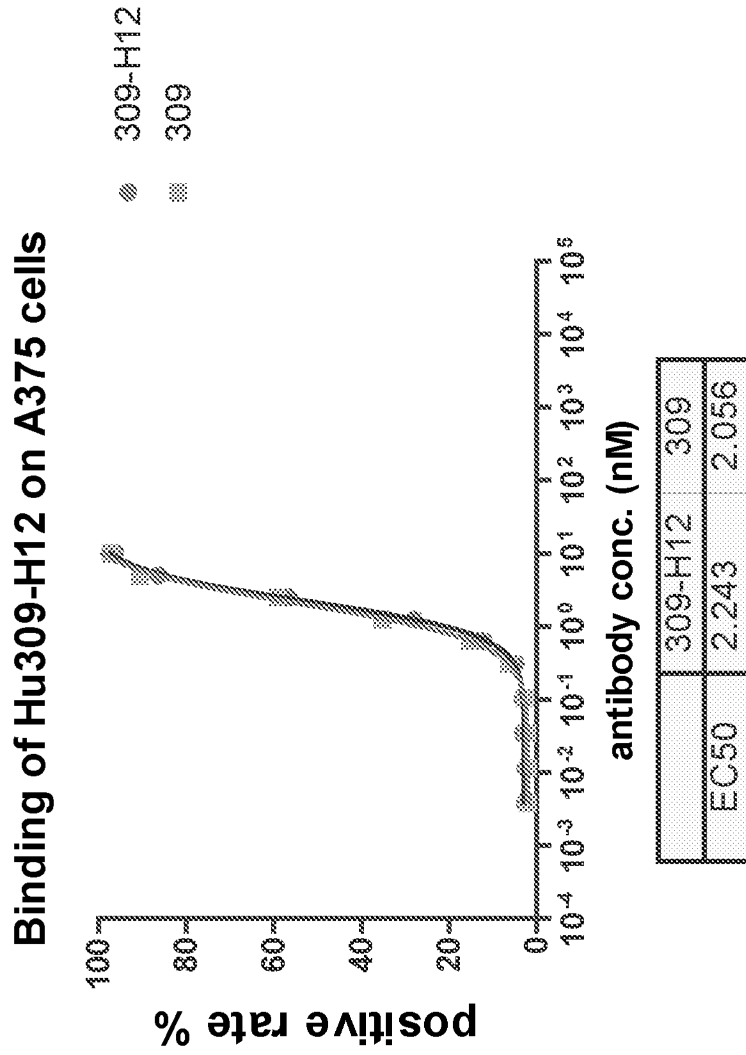


FIG. 2



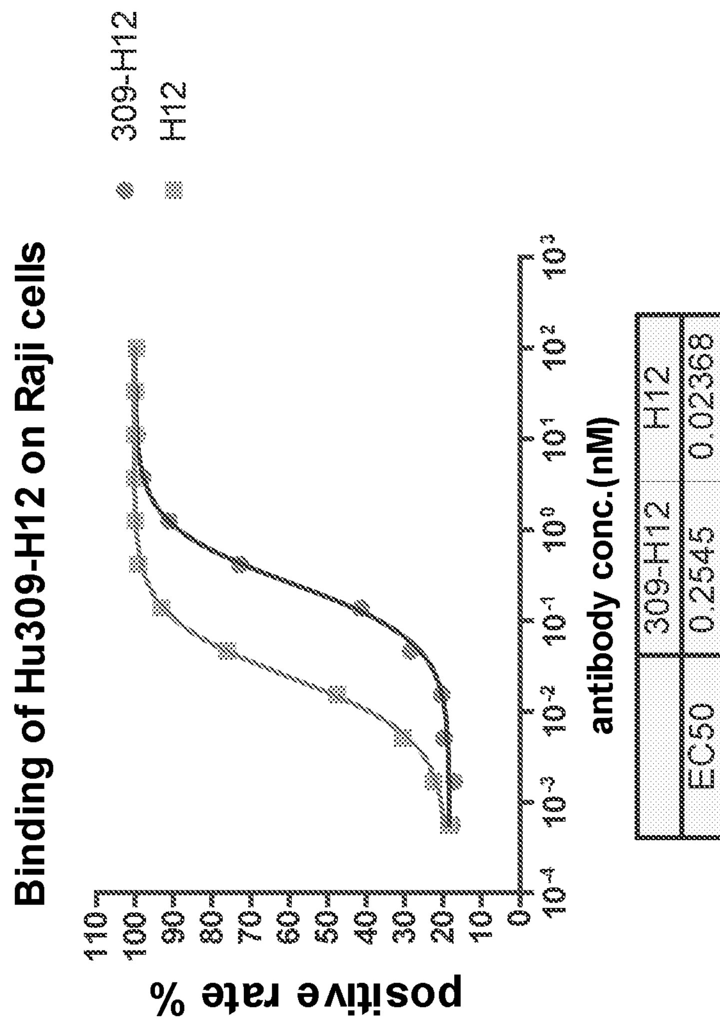
	H12(PDL1)	309-H12
EC50	9.484	179.2

FIG. 3



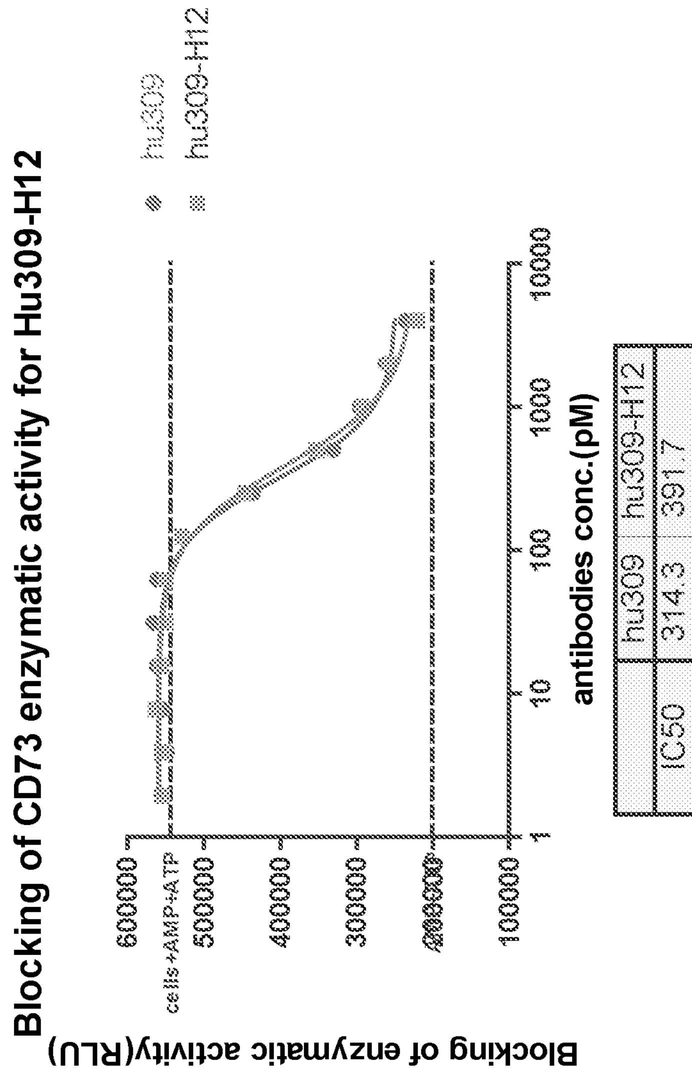
	309-H12	309
EC50	2.243	2.056

FIG. 4



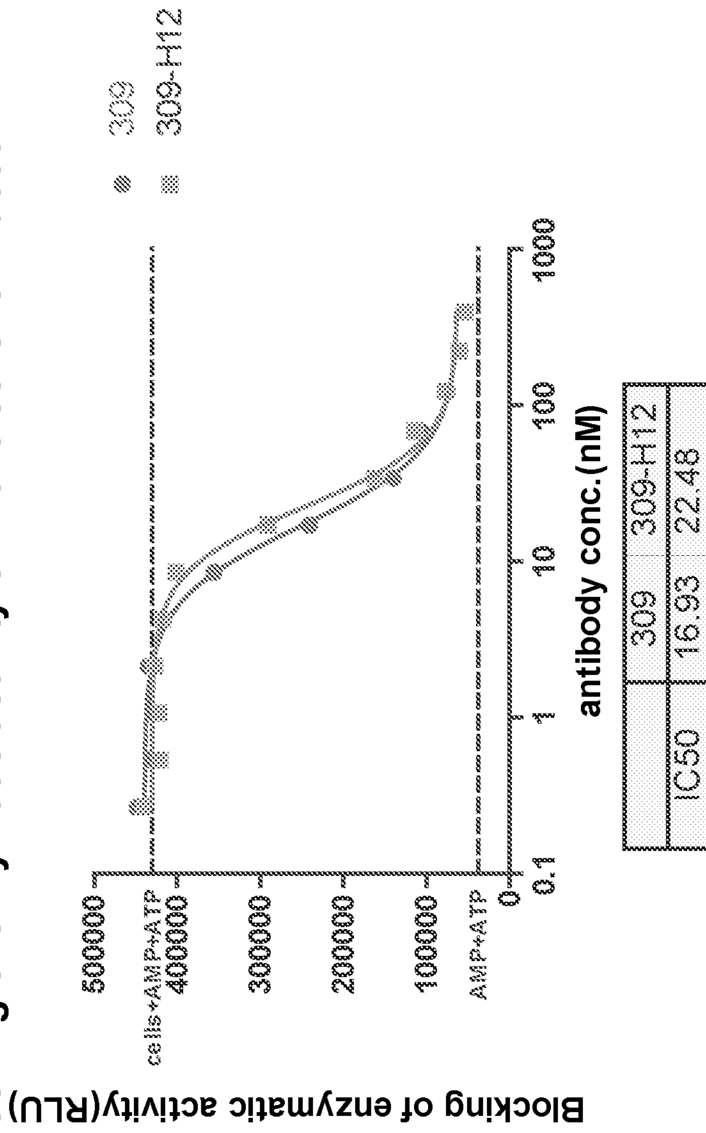
	309-H12	H12
EC50	0.2545	0.02368

FIG. 5



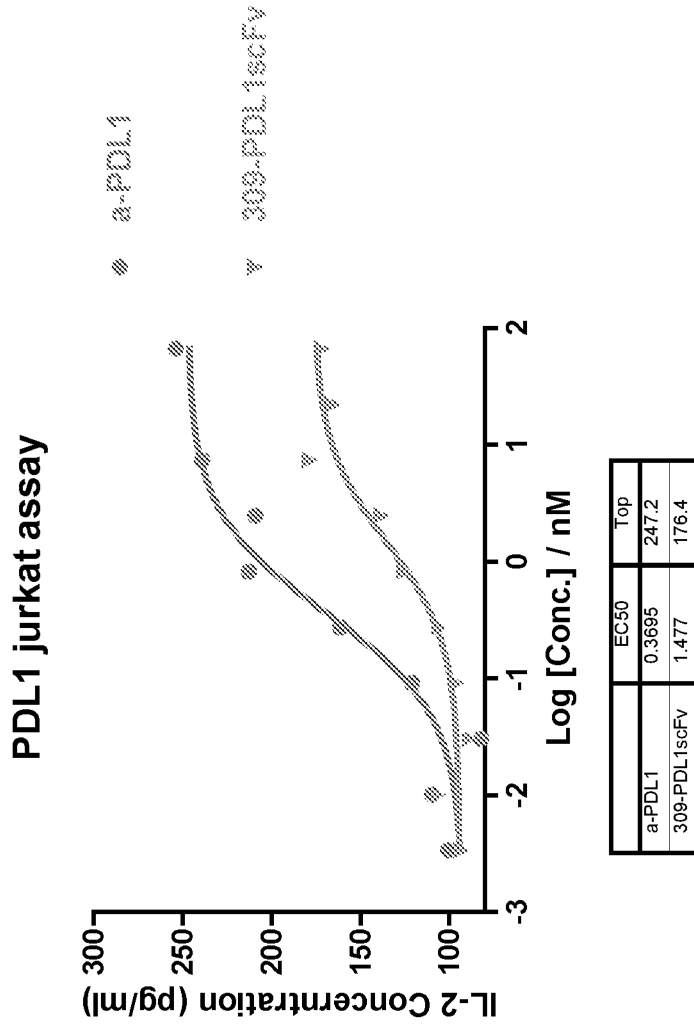
	hu309	hu309-H12
IC50	314.3	391.7

FIG. 6



	309	309-H12
IC50	16.93	22.48

FIG. 7



	EC50	Top
a-PDL1	0.3695	247.2
309-PDL1scFv	1.477	176.4

FIG. 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2019/097774

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/28(2006.01)i; C07K 16/46(2006.01)i; A61K 39/39(2006.01)i; A61P 35/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K, A61K, A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

VEN, CNABS, PubMed, CNKI, ISI Web of Knowledge, Google: CD73, 5'-NT, PD-L1, Programmed death-ligand 1, B7-H1, CD73/PD-L1, anti-CD73 x PD-L1, bispecific, bispecific antibody, BsAb, BiTEs, complex, diabodies, cancer, tumor, T-cell, activation, SEQ ID NO: 1-12

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PA	WO 2018137598 A1 (I-MAB) 02 August 2018 (2018-08-02) the whole document	1-32
A	WO 2017220989 A1 (KYMAB LIMITED) 28 December 2017 (2017-12-28) the whole document	1-32
A	CN 107488229 A (ZANG, J.W.) 19 December 2017 (2017-12-19) the whole document	1-32
A	WO 2017220990 A1 (KYMAB LIMITED) 28 December 2017 (2017-12-28) the whole document	1-32
A	WO 2016081748 A2 (BRISTOL-MYERS SQUIBB COMPANY) 26 May 2016 (2016-05-26) the whole document	1-32
A	WO 2017218435 A1 (ASKGENE PHARMA INC.ET AL.) 21 December 2017 (2017-12-21) the whole document	1-32
A	SÁNCHEZ -PAULETE, A.R.et al. "Cancer immunotherapy with immunomodulatory anti-CD137 and anti-PD-1 monoclonal antibodies requires Batf3-dependent dendritic cells" *Cancer Discov.*, Vol. 6, No. 1, 31 January 2016 (2016-01-31), pages 71-79	1-32

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 October 2019	**30 October 2019**

Name and mailing address of the ISA/CN	Authorized officer
National Intellectual Property Administration, PRC **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088** **China**	**DAI,Yuehan**
Facsimile No. **(86-10)62019451**	Telephone No. **86-(010)-53962039**

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TERP，M.G.et al. "Anti-Human CD73 Monoclonal Antibody Inhibits Metastasis Formation in Human Breast Cancer by Inducing Clustering and Internalization of CD73 Expressed on the Surface of Cancer Cells" *The Journal of Immunology,* Vol. 191, 16 September 2013 (2013-09-16), pages 4165-4173	1-32
A	JI，S.Y.et al. "Bispecific antibodies and their application in lung cancer treatment" *Academic Journal of Chinese PLA Medical School,* Vol. 38, No. 9, 30 September 2017 (2017-09-30), pages 883-885	1-32

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: **24, 26-30**
because they relate to subject matter not required to be searched by this Authority, namely:

   [1] Although claims 24, 26-30 direct to a method of treatment of the patients, the search has been carried out and based on the use of the derivative or pharmaceutical composition for the manufacturing of a medicament for the treatment of diseases.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2019/097774

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
WO	2018137598	A1	02 August 2018	EP	3383916	A4	11 September 2019
				US	2019256598	A1	22 August 2019
				KR	20180093088	A	20 August 2018
				AU	2018213549	A1	06 June 2019
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WO	2017220989	A1	28 December 2017	EP	3471754	A1	24 April 2019
				WO	2017220990	A1	28 December 2017
				BR	112018076260	A2	26 March 2019
				EP	3471753	A1	24 April 2019
				WO	2017220988	A1	28 December 2017
				KR	20190032355	A	27 March 2019
				CA	3026474	A1	28 December 2017
				AU	2017281830	A1	13 December 2018
				TW	201803904	A	01 February 2018
				CN	109475602	A	15 March 2019
				TW	I640536	B	11 November 2018
				SG	11201810509P	A	28 December 2018
				KR	20190030208	A	21 March 2019
				WO	2017220990	A9	01 March 2018
				TW	201900680	A	01 January 2019
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				WO	2017220988	A9	15 February 2018
				WO	2017220989	A8	27 December 2018
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WO	2017220990	A1	28 December 2017	EP	3471754	A1	24 April 2019
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				KR	20190030208	A	21 March 2019
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				TW	201900680	A	01 January 2019
				IL	263834	D0	31 January 2019
				WO	2017220988	A9	15 February 2018
				WO	2017220989	A8	27 December 2018

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2019/097774

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
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		US 2018127513 A1	10 May 2018
		TN 2017000203 A1	19 October 2018
		AR 102698 A1	15 March 2017
		SG 11201703192S A	30 May 2017
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		CL 2017001296 A1	16 February 2018
		KR 20170080699 A	10 July 2017
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		PH 12017500918 A1	20 November 2017
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		TW 201625693 A	16 July 2016
		PE 07822017 A1	04 July 2017
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WO 2017218435 A1	21 December 2017	CN 109414500 A	01 March 2019
		US 2017355770 A1	14 December 2017
		US 10077308 B2	18 September 2018